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ADF

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FINAL REPORT

20030619

ANIMAL DISEASE DIAGNOSTICS

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ANIMAL DISEASE DIAGNOSTICS
FINAL REPORT
PROJECT: 20030619

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ABSTRACT

The objectives of Animal Disease Diagnosis research were to develop and validate new or improved diagnostic tests for animal diseases that are needed by livestock industry in Saskatchewan. This project (ADF 20030619) was limited to providing bridging finances for the Western College of Veterinary Medicine (WCVM) in salary support for the applicant for a period of one year following the termination of the ADF Strategic Research Program. This funding has allowed the WCVM to retain essential molecular diagnostic expertise for research, diagnostic service and teaching. Projects related to Animal Disease Diagnostics, whose operational costs have been funded by other agencies, have been permitted to continue through this one-time salary support from the ADF.

TECHNICAL REPORT

This report contains the results from 5 animal disease diagnosis research activities. 1) Livestock Disease Diagnosis Test Development. 2) Development of Diagnostic Test Methods for Detecting Animal Pathogens in Bacteria-Containing Bio-degradation Products. 3) WCVM Disease Investigations. 4) Role of BVD virus in undifferentiated fever of feedlot cattle. 5) Molecular tools for the characterization of *Mycobacterium avium* subspecies *paratuberculosis*.

Activity 1) Livestock Disease Diagnosis Test Development FUNDED by ADF 20020269.

The objective for Activity 1 was to establish disease diagnostic testing protocols aimed at accuracy, speed and low cost to the producer. These tests are essential to support this provinces growing agricultural sector by lowering the cost of disease and by protecting international markets. In the past year we have utilized new molecular biotechnology to improve livestock infectious disease diagnostic testing for an important disease-causing bacteria of cattle, pigs and poultry, and two viruses of goats and deer which are closely-related to (and could be confused with) similar viruses in cattle and sheep. Three other tests for bacteria causing disease in cattle and pigs were discontinued due to lack of operational funds.

ADF-Project Report	
Pasteurella multocida toxin gene identification	DONE
Pasteurella multocida capsule typing	DONE
Caprine and cervid herpes viruses	DONE

Mycoplasma synoviae Stopped
Mycoplasma bovis Stopped
Ureaplasma sp. Stopped

Pasteurella multocida is a species of bacteria that cause septicemic or respiratory disease in domesticated and wild animals and birds. *P. multocida* compounds respiratory diseases caused by viruses and other bacteria. It can be found in the upper respiratory tract (nose, throat, & windpipe) of most cattle. *Pasteurella* infection of the upper respiratory is thus very common, even in normal healthy calves. *Pasteurella* infections are spread by inhalation of aerosol droplets, by direct nose to nose contact, or by ingestion of feed and water contaminated by nasal and oral discharges from infected cattle; hence the bacteria are easily spread between cattle, especially when calves are crowded (as in shipment) or closely confined (as in a dairy calf nursery). The animal's normal body defences keep the *Pasteurella* infections in check; the bacteria reproduce at a slow rate, float around in the mucus of the nose and throat, are destroyed by locally produced antibodies, and are easily removed by a clearing mechanism that is found in healthy animals.

Pasteurellosis disease occurs when the animal's normal defences are compromised. In this case the lining of the upper respiratory tract could be damaged by IBR, PI-3, or BRSV viruses, the clearing mechanism could be depressed by BVD virus, or the production of local antibodies against *Pasteurella* may be interrupted by environmental or nutritional stress. When the defences are compromised, the bacteria become attached to the lining of the respiratory tract (colonize), reproduce rapidly, and spread throughout the lungs. The severity of the disease depends upon which Pasteurella organism is involved and, at least in calves, with the nature of associated infections (IBR, PI-3, BVD, BRSV, or other bacteria).Diagnostic tests to identify bacteria-strains based on genes for important factors that are associated with virulence are a fundamental tool for disease investigation studies. For example, the presence of *P. multocida* is not an unexpected finding, but identifying a strain which contains one or several toxin genes and particular cell wall antigens aids in the interpretation of significance of the association of that bacteria with the disease.

Malignant Catarrhal Fever (MCF) is a fatal and multisystemic, herpesviral disease affecting many domestic and wild ungulates. Ovine herpes virus-2 (OHV-2) is found, and causes no disease, in healthy domestic sheep, but causes severe or fatal disease in other susceptible domestic and wild ungulates. Recently, two new forms of the virus were reported. In 2000 a new virus was identified in diseased white-tailed deer (WTD) and in 2002, another version of the herpes virus was described in goats. Some animals can be infected with the virus for extended periods of time before showing clinical signs of MCF while some animals test positive for the viral antibody, but show no clinical signs and in some cases, animals are found dead with no clinical signs ever observed. Complete understanding of the herpes virus has been elusive. A test for caprine and WT-deer forms of the herpes virus were important to differentiate illness or deaths caused by these viruses from the sheep-form, ovine herpes virus-2.

Methods:

We developed 4 different laboratory protocols by adapting or designing test-specific reagents to be used to amplify specific genomic sequences. Polymerase Chain Reaction (PCR) is

a molecular biotechnology technique which makes possible methods for highly sensitive detection of specific genetic sequences. Diagnostic test development takes place in two phases. Phase one, optimization, involves a feasibility study to ensure that the needs of the industry for any test are known prior to development. This is followed by development, optimization and standardization of a suitable methodology and preparation of a working-protocol. The validation phase determines assay performance through testing of samples known to known positive or negative for the presence of the pathogen of interest. Following these phases, the test can be adopted by Prairie Diagnostic Services Inc by technology transfer.

Results:

The measurable output of test development is an optimized protocol suitable for validation and diagnostic testing. Described below are the four optimized protocols.

PASTEURELLA MULTOCIDA CAPSULE TYPING

The first priority in this project is a PCR technique to identify the presence of toxin in Pasteurella multocida. We have finished optimizing the test and are seeking field samples for validation.

* Reference: Townsend et al. (Journal of Clinical Microbiology, 2001, 39, 924-929)
* Sample extraction protocol: DNA Extraction protocol (PK) - see standard protocol section
* Sample source: cultured organism
* Genes amplified: capsule serogroup genes

Primer Design

Primer KMT1T7	Tm = 70EC	ATCCGCTATTTACCCAGTGG
Primer KMT1SP6	Tm = 71EC	GCTGTAAACGAACTCGCCAC
Primer CAPA-F	Tm = 69EC	TGCCAAAATCGCAGTCAG
Primer CAPA-R	Tm = 63EC	TTGCCATCATTGTCAGTG
Primer CAPB-F	Tm = 66EC	CATTTATCCAAGCTCCACC
Primer CAPB-R	Tm = 68EC	GCCCGAGAGTTTCAATCC
Primer CAPD-F	Tm = 72EC	TTACAAAAGAAAGACTAGGAGCCC
Primer CAPD-R	Tm = 71EC	CATCTACCCACTCAACCATATCAG
Primer CAPE-F	Tm = 68EC	TCCGCAGAAAATTATTGACTC
Primer CAPE-R	Tm = 69EC	GCTTGCTGCTTGATTTTGTC
Primer CAPF-F	Tm = 74EC	AATCGGAGAACGCAGAAATCAG
Primer CAPF-R	Tm = 72EC	TTCCGCCGTCAATTACTCTG

Product size: KMT1 - 460 bp, CapA - 1044 bp, CapB - 760 bp, CapD - 657 bp, CapE - 511 bp, CapF - 851 bp

Optimized reaction conditions

PCR Reagents		Thermal cycler conditions		
Water	29.25 ul	Initial denature	94C	5 min
Buffer	5.0 ul	Denature	94C	30 s
Mg^{2+}	7.0 ul	Anneal	55C	30 s
Primers (each)	3.0 ul	Elongation	72C	30 s
dNTPs	0.5 ul	Cycle number	30	
Taq polymerase	0.4 ul	Finishing	72C	10min
sample DNA	2.0 ul	Soak	10C	

PASTEURELLA MULTOCIDA TOXIGENICITY

The first priority in this project is a PCR technique to identify the presence of toxin in Pasteurella multocida. We have finished optimizing the test and are seeking field samples for validation.

* Reference: Buys et al. (Nucleic Acids Res., 1990, 18, 2815-2816), Petersen (Mol. Microbiol., 1990, 4, 821-830), Lax et al. (FEBS Lett., 1990, 277, 59-64)
* Sample extraction protocol: DNA Extraction protocol (PK) - see standard protocol section
* Sample source: cultured organism
* Gene amplified: toxin gene

Primer Design

Primer ToxA-F	Tm = 61EC	CTT AGA TGA GCG ACA AGG
Primer Tox A-R	Tm = 60EC	GAA TGC CAC ACC TCT ATA G
Product size = 831 bp		

Optimized reaction conditions

PCR Reagents		Thermal cycler conditions		
Water	38.75 ul	Initial denature	95C	5 min
Buffer	5.0 ul	Denature	95C	30 s
Mg^{2+}	2.0 ul	Anneal	55C	30 s
Primers (each)	1.0 ul	Elongation	72C	30 s
dNTPs	0.25 ul	Cycle number	35	
Taq polymerase	0.25 ul	Finishing	72C	10min
sample DNA	2.0 ul	Soak	10C	

CAPRINE HERPES nPCR PROTOCOL

The first priority in this project is a PCR technique to identify the presence of the virus. We have finished optimizing the test and are seeking field samples for validation.

* Reference: Li etal. J Vet Diagn Invest 2003, 15: 46-49.
* Sample extraction protocol: DNA Extraction protocol (PK) - see standard protocol section
* Sample source: cultured organism
* Gene amplified: toxin gene

Primer Design

Primer CON-EX-F:	Tm = 57-62EC	CAYAAYCTRTGCTACTCCAC
Primer CONS-F:	Tm = 81EC	TGGCCTCGGGCATGCTGC
Primer CpHV-r:	Tm = 64EC	CCGTAATAGAGGGGTCCT

Product size = CONS-F & CpHV-r - 144bp

Optimized reaction conditions

Primary PCR		Thermal cycler conditions (CpHV2-1)		
PCR Reagents		Initial denature	94C	5 min
Water	31.00 ul	Denature	94C	30 s
Buffer	5.0 ul	Anneal	46C	60 s
Mg^{2+}	2.0 ul	Elongation	72C	60 s
DMSO	1.25 ul	Cycle number	45	
Primers (each)	2.5ul CON-EX-F & CpHV-r	Finishing	72C	10min
dNTPs	0.5 ul	Soak	10C	
Taq polymerase	0.5 ul			
sample DNA	5.0 ul			
Secondary PCR		Thermal cycler conditions (CpHV2-2)		
PCR Reagents		Initial denature	94C	5 min
Water	36.00 ul	Denature	94C	30 s
Buffer	5.0 ul	Anneal	46C	30 s
Mg^{2+}	2.0 ul	Elongation	72C	30 s
DMSO	1.25 ul	Cycle number	35	
Primers (each)	1.5ul CONS-F & CpHV-r	Finishing	72C	10min
dNTPs	0.5 ul	Soak	10C	
Taq polymerase	0.25 ul			
sample DNA	2.0 ul			

DEER HERPES nPCR PROTOCOL

Primary PCR

Station A - master mix preparation laminar flow hood

28.5 ul sterile ultra-pure water
5 ul 10x PCR Buffer
4 ul (25 mM) $MgCl_2$
2.5 ul (2 x 2.5 ul 20 pmol) each primer CON-EX-F & der-r
1.5 ul DMSO
0.5 ul dNTPs (25 mM)
0.5 ul (5 U/ul) Taq polymerase

Dispense 45 ul of master mix into each PCR tube. Overlay with 1 drop of mineral oil.

Station B - Sample Addition laminar flow hood

Dispense 5 ul DNA into tube lid. Cap tubes tightly. Centrifuge tubes for 30s. Immediately place tubes into pre-heated thermal cycler. (Herpes1)

Secondary PCR

Station A

31.5 ul sterile ultra-pure water
5 ul 10x PCR Buffer
4 ul (25 mM) $MgCl_2$
2.5 ul (2 x 2.5 ul 20 pmol) each primer CONS-F & der-r
1.5 ul DMSO
0.5 ul dNTPs (25 mM)
0.5 ul (5 U/ul) Taq polymerase
Dispense 48 ul of master mix into each PCR tube. Overlay with 1 drop of mineral oil.

Station B

Dispense 2 ul of primary PCR sample into tube lid. Cap tubes tightly. Centrifuge tubes for 30s. Immediately place tubes into pre-heated thermal cycler. (Herpes1)

Station C - Electrophoresis area

Visualize PCR amplicons in 1.25% agarose gel with ethidium bromide staining. Record results with AlphaImager gel documentation camera.

Thermal Cycler Programme: Herpes1

94EC - 5 min
94EC - 30 s } x 45
46EC - 60 s }
72EC - 60 s }
72EC - 10 min
10EC - forever
End

Secondary Primer set: CONS-F & DER-r - 155bp
Primer Sequences: CON-EX-F: CAY AAY CTR TGC TAC TCC AC
CONS-F: TGG CCT CGG GCA TGC TGC
DER-r: GGG CGA TTG ACT CTT TAT AA

Activity 2) Development of Diagnostic Test Methods for Detecting Animal Pathogens in Bacteria-Containing Commercial Bio-degradation Products. FUNDED by Environment Canada.

Bacteria-containing commercial products, sold for bio-degradation of human and animal wastes, consist of complex mixtures of bacteria. These mixtures are often of undetermined composition and grown in batch cultures from diverse bacteria-rich inocula of proprietary origins. In order to provide a means of testing for the presence of small numbers of microorganisms pathogenic to terrestrial or avian animals, in bacteria-containing bio-degradation products, we developed 15 microorganism-specific polymerase chain reaction-based (PCR) assays. A method was optimized to extract total genomic DNA from nine bio-degradation products of different formulations (solid, liquid, powder). Each PCR assay was demonstrated to be specific, as no cross-reactions were identified when tested with DNA from 82 other species of bacteria. We were able to assess the mean detection limit for 10 assays using cultured organisms spiked into bio-degradation products. The mean was $1x10^{2.62} \pm 1x10^{1.58}$ CFU/g (bacteria) and $1x10^{3.88} \pm 1x10^{1.14}$ cells/g (protozoa). This study has demonstrated that molecular diagnostic means can be used to detect small numbers of selected animal pathogens in complex bio-degradation products. There were no animal pathogens detected by the PCR assays in unspiked bio-degradation products.

Bacteria-containing commercial products, sold for bio-degradation of human and animal wastes can be purchased in commercial retail stores in a variety of different solid, liquid and powdered formulations. Use of these products in close proximity to livestock as well as aquatic, terrestrial and avian animals could lead to the potential for deleterious effects on animal health. Of particular concern is the risk that some bacteria-based products, derived from environmental sources, such as municipal or livestock wastes, may also contain pathogenic organisms which have survived or even proliferated in the manufacturing process. In order to assess the safety of these products, there is a need for suitable diagnostic test procedures capable of detecting potentially small numbers of selected pathogenic bacteria contaminating rich cultures of benign bacteria. We developed a molecular-diagnostic approach to the detection of important bacterial and protozoan pathogens in nine examples of bacteria-based bio-degradation products.

This section of the report describes the development of a DNA extraction protocol suitable for purifying DNA from a variety of bacteria-containing commercial products, as well as the development of polymerase chain reaction (PCR) protocols for the detection of selected pathogens. The initial study demonstrated that an optimized DNA extraction protocol combined with an appropriately specific PCR assay could be used successfully to test a wide range of bio-degradation products regardless of composition or formulation.

The second phase of the study was to determine minimum detection limits for selected bacterial and protozoan pathogens when spiked into examples of a variety of bacteria-containing commercial bio-degradation products. The selected pathogens were *Campylobacter* sp., *Clostridium piliforme*, *Clostridium botulinum* Types C and E, *Cryptosporidium parvum*, *Francisella tularensis*, *Giardia lamblia*, *Mycoplasma gallispeticum*, *Mycobacterium avium*, *Mycobacterium bovis*, *Pasteurella multocida*, *Reimerella anatipestifer*, *Salmonella* sp., *Toxoplasma gondii*.

Activity 3: Support for Diagnostic Disease Investigations. FUNDED by individual WCVM researchers.

Traditional diagnostic test methods are known to have poor sensitivity for fastidious or unculturable organisms and an inability to detect organisms that fail to elicit detectable host immune responses. In veterinary medicine, as in human medicine, such limitations are common and this leads to cases of infectious disease for which no etiologic diagnosis can be found. Frequently, disease investigation require the creation of a new test capable of identifying unknown organisms. In the past year, seven requests of this kind were made (see list below).

Disease Investigations:

Avian pox virus (Smits)	DONE
Blastomyces sp. (Skuba)	DONE
Fusobacterium sp. (Chirino)	DONE
Unexplained calf diarrhea (Waldner)	On-going
Equine herpes in a donkey (Haines)	On-going
Pelican histoplasma-like protozoans (Wobeser)	Done
Mycobacterium in bison (Woodbury/Chirino)	Initiated

The diagnostic laboratory system is limited in its ability to identify unknown organisms and a proposal to develop a new class of molecular tests specifically for this purpose was submitted to ADF. Such tests would provide an essential ability to rapidly identify emerging infectious diseases and safe-guard from losses caused by unknown organisms.

Activity 4: Role of BVD virus in undifferentiated fever of feedlot cattle. A collaborative Research Project. FUNDED by Alberta Livestock Industry Development Fund to Dr C. Booker - Feedlot Health Management Systems, Okotoks, Alberta.

Undifferentiated fever (UF) or bovine respiratory disease complex (BRD) continues to be the single most important infectious disease entity in beef feedlot production. Current management practices have focussed on successfully managing this disease complex through the use of prophylactic and therapeutic antimicrobial strategies. However, there are substantial amounts of concrete and circumstantial epidemiologic, pathologic, serologic and immunologic evidence of an association between bovine viral diarrhea virus (BVDV) and the UF/BRD complex. Unfortunately there is limited data that describe s how transmission of BVDV in the feedlot occurs and how this transmission leads to the development of UF/BRD. The purpose of this project is to improve our understanding of the role of BVDV in pathogenesis of pen-level UF/BRD. From the laboratory perspective, appropriate BVDV diagnostic tests will be developed to allow for the cost-effective identification of BVDV and differentiation between immunotolerant persistently infected and immunocompetent acutely infected animals.

This project is ongoing. A molecular PCR-based test was developed, optimized and employed on this project. This test has the benefit of being able to differentiate between type1 and type 2 BVD viruses. In the first year of the two year project 5000 samples were tested using a pooling scheme which reduced costs by 77% over testing every sample. The design of the experiment included 2 feedlots, one had a high prevalence of persistently infected animals and the other had low. There was a higher mortality rate at the high BVD prevalence feedlot, suggesting a positive correlation. In addition, 58% of the persistently BVD infected animals died

prior to slaughter and there was some evidence that the persistently infected animals were transmitting the virus to other animals within pens. This information, when backed with data from the second year of the study will be important in designing new feedlot management practices to reduce UF/BRD.

Activity 5) Development of molecular epidemiological tools for the characterization of polymorphisms among isolates of *Mycobacterium avium* subspecies *paratuberculosis*. FUNDED by AFIF

Genetic typing of micro-organisms is fundamental to the understanding of bacterial genome organization and microbial evolution. Practical methods that distinguish among clinical isolates, different species and between virulent and non-virulent strains are critical to our understanding the transmission of pathogenic micro-organisms among human and animal populations.

Genetic typing or strain typing of micro-organisms may provide insight into their epidemiology, aiding in disease prevention and control. For example, the discovery of a gene existing only in certain strains may be related to the mechanisms of disease or antibiotic resistance. The same molecular techniques used for strain typing can also be applied to host genomes and can be used to investigate why some hosts are more susceptible to disease than others.

DNA amplification has provided a means of analyzing micro-organisms for the presence, size and copy number of any given gene or nucleotide sequence. Additional molecular techniques can be used to detect variations in genes or sequences among microbial isolates of the same species.

Several strain typing techniques are available to categorize *Mycobacterium avium* subspecies *paratuberculosis* (*M. paratuberculosis*) isolates into 4 main groups (cattle, sheep, bison, intermediate). However the majority of isolates are identified as members of the Cattle group, regardless of host origin. This suggests that the cattle group of *M. paratuberculosis* isolates are very successful because host specificity is not a variable or the small differences required to demonstrate host specificity have not yet been found.

It is known that polymorphic or variable regions of a bacterial genome can be targeted to display intra-species heterogeneity. These variable regions can be used to portray the crucial genetic differences between isolates to allow a more significant epidemiological analysis. The primary goal of research presented in this thesis was to find the appropriate molecular tools to identify additional polymorphic regions of the *M. paratuberculosis* genome. The specific objectives were: (1) Collect *M. paratuberculosis* isolates from a broad range of ruminant hosts and geographic locations. (2) Adopt published strain typing protocols to identify isolates that fall into the established Bison, Cattle, Sheep and Intermediate groups. (3) Identify polymorphic or variable regions which could be used to further subdivide isolates into epidemiologically useful groups.

M. paratuberculosis has been described as extremely homogenous (104), therefore finding genetic polymorphisms that can distinguish between epidemiologically significant isolates or facilitate a new diagnostic test are of great interest. Once a functional polymorphic region has been identified, an assay can be designed to exploit that polymorphism. The

discriminatory power of this assay is of particular importance, especially for when targeting organisms with limited genetic diversity, such as *M. paratuberculosis*.

Our current knowledge of strain typing has demonstrated the existence of cattle, sheep and bison-related strains of *M. paratuberculosis*. Among the available *M. paratuberculosis* typing assays, RFLP, PFGE and AFLP seem to be the most reproducible with the highest discriminatory value. Unfortunately, a comparison of discriminatory powers of AFLP versus PFGE versus RFLP, for *M. paratuberculosis* typing, is not available. In this study, RFLP was attempted over AFLP because it is the most widely used strain typing technique for *M. paratuberculosis* typing and was therefore needed as a comparison to any newly developed techniques.

Several studies have made reference to the difficulties associated with cultivating sheep isolates (120,169,170) and how this impacts genotyping studies which require the growth of *M. paratuberculosis* isolates. The majority of the isolates analysed in this study could not be cultivated in the liquid media required for both RFLP and PFGE, which limits the usefulness of these assays. On top of the growth requirements, both RFLP and PFGE assays require extensive preparation time and therefore results can take many months, again limiting the usefulness of these assays.

An analysis of different mini- and micro-satellite regions resulted in 1 region where genetic differences could be detected among the *M. paratuberculosis* isolates analyzed in this study. The satellite typing assay described in this publication closely parallels that of an RAPD assay. *In silico* analysis of the *M. paratuberculosis* genome provided evidence for a single locus, 22 base pair repeat region. However, more than one region of the *M. paratuberculosis* genome was amplified by PCR. There are 2 explanations for these results. First, only 1 genomic nucleotide sequence of *M. paratuberculosis* is available, therefore *in silico* analysis is only accurate for this single sequenced isolate. Second, the primers designed to amplify the 22 base pair repeat region could be homologous to other regions of the *M. paratuberculosis* genome.

Regardless of the explanation, these results support the theory that there is more variation among *M. paratuberculosis* isolates than what is currently being detected. SSCP analysis was demonstrated to be a rapid and reliable technique for the screening of *M. paratuberculosis* isolates for sequence polymorphisms which could be further targeted for diagnostic or typing purposes. The benefits of SSCP analysis are the lack of requirement of live organisms or large amounts of high quality genomic DNA. SSCP analysis could rapidly type all *M. paratuberculosis* isolates, regardless of host or sample origin. Unfortunately, the regions analyzed by SSCP in this study do not provide the high discriminatory power associated with RFLP, AFLP or PFGE. But the results of this study indicate that SSCP analysis can be used as a beneficial tool for finding an appropriate polymorphic region within the *M. paratuberculosis* genome.

Collaborative Projects related to Animal Disease Diagnosis 2003/2004

1) *Campylobacter fetus* subspecies identification (Dr Devenish, CFIA). Stopped
We screened putative *C. fetus fetus* (n= 57) and *C. fetus venerealis* (n= 23) cultures for single nucleotide polymorphisms in the *Cpn60* gene. The SRP pattern agreed with culture results (96.4% agreement).

2) Identification of Mycobacterium avium paratuberculosis (Gov NWT). Ongoing.
Several free-range herds of wild bison in the NWT may, on the basis of previous culture and PCR results, be infected with the agent for Johne's disease. I have been contracted to do further testing of fecal samples for this pathogen and this work should be completed by year's end.

3) Pattern Recognition and Pathology Informatics (Dr Kusilik and Dr Eramian, Dept of Computing Sciences) Initiated
Describe tools and technologies for structuring and analysing pathology data and their applications in pathology informatics. Evaluate currently available pattern-recognition algorithms for analysis of histology slides.

4) Strongyl-nematode identification (Dr Polley/ Kutz). Ongoing
A new species of strongyl-nematode was discovered in Caribou. It appears to be phylogenetically related to *Varastrongylus alpenae*.

5) Trypanosoma sp. identification (Dr Kutz). Ongoing
Caribou have been found to harbour Trypanosomes in their blood. Sequence analysis suggests it is not *T. theileri*. No sequence is available for comparison with *T. cervi*.

6) Quantifying *Clostridium botulinum* spores in soil samples (Bollinger) Ongoing
A PCR was developed to identify the C-type toxin gene. We plan to develop another assay for *C. botulinum* (probably 16S rDNA) and we plan to investigate spore concentration and DNA extraction techniques to allow the test to be performed without the need for liquid culture.

PROJECT PERSONNEL

Personnel associated with Animal Disease Diagnosis projects funded by other agencies:

Name	Funding Agency
Anita Quon	Environment Canada
Rebecca Cross	Environment Canada
Kara Dutcyvich	Environment Canada
Jennifer Sibley	AFIF Chair in Alternative Livestock, BC Cattleman's Association's Brigadier WN Bostock Memorial Research Grant.
Brendan Clancy	Alberta Livestock industry Development Fund
Kerry Bridges	Alberta Livestock industry Development Fund
Anju Tumber	Prairie Diagnostic Services
Janice Woodcock	Prairie Diagnostic Services

EQUIPMENT

No equipment was purchased or rented with project funds.

PROJECT DEVELOPED MATERIALS 2003-2004

A copy of these are on the accompanying CD or are attached in hardcopy.

Posters and Presentations at Conferences (presenter underlined)

Lorry B. Forbes, **Greg D. Appleyard**, Alvin A. Gajadhar. Use of a synthetic antigen in an ELISA for the serological diagnosis of trichinellosis in swine. 3rd Annual Meeting of the Canadian Animal Health Laboratorians Network (CAHLN), May 16-24, 2004, Guelph, Ontario.

K. McIntosh, J. Harding, J. Ellis, **G. Appleyard**. Nested PCR detection & duration of Porcine Circovirus Type 2 in semen from naturally infected boars. American Association of Veterinary Laboratory Diagnosticians, Greensboro, Oct 2004.

G.D. Appleyard, Animals Disease Diagnostics and Pathogen Discovery. Virtual College of Biotechnology. University of Saskatchewan. January 2004.

E Jenkins, A Veitch, **GD Appleyard**, E Hoberg, D Ogunremi, S Kutz, L Polley. A newly recognized neurological disease associated with *Parelaphostrongylus odocoilei* in experimentally infected thinhorn sheep. 53rd Annual Meeting of the Wildlife Disease Association. August, 2004. San Diego, CA

S Kutz, **G D Appleyard**, E Jenkins, A Scheer, J Nagy, A Veitch, B Elkin, D Cooley, D Johnson, E Hoberg, L Polley. Assessing, Predicting, and Monitoring the Impact of Climate Change on

Infectious Diseases in Caribou: What Do We Know and What Do We Need to Know? 10th North American Caribou Conference. Alaska. May 2004.

JL Davies, JA Sibley, EG Clark, and **GD Appleyard**. Histologic and genotypic characterization of cutaneous mycobacteriosis in cats: a retrospective study on formalin-fixed and paraffin embedded tissues. American Association of Veterinary Laboratory Diagnosticians, San Diego, Oct 2003.

JA Sibley and **GD Appleyard**. Detection of selected wildlife pathogens in commercial waste digestion products containing bacteria. American Association of Veterinary Laboratory Diagnosticians, San Diego, Oct 2003.

KA McIntosh, JCS Harding, JA Ellis, and **GD Appleyard**. Detection of porcine circovirus type 2 viremia and seroconversion in swine. American Association of Veterinary Laboratory Diagnosticians, San Diego, Oct 2003.

Publications (Accepted / Submitted) 2003-2004

1) Jenkins E, **Appleyard, GD**, Hoberg E, Rosenthal B, Kutz S, Veitch A, Schwantje H and Polley L. Molecular identification and geographic distribution of the muscle-dwelling nematode *Parelaphostrongylus odocoilei* in North America. Accepted in J Parasitology 2004.

2) JA Sibley, RH Cross, AL Quon, K Dutcyvich, TA Edge, FA Leighton, and **GD Appleyard**. Development of Diagnostic Test Methods for Detecting Key Wildlife Pathogens in Bacteria-Containing Commercial Bio-degradation Products. Accepted in World Journal of Microbiology and Biotechnology 2004.

3) KE Kewish, **Appleyard, GD**, S Meyers, BA Kidney & ML Jackson. *Mycoplasma haemofelis* and *Mycoplasma haemominutum* detection by polymerase chain reaction in cats from Saskatchewan and Alberta. Can Vet J. 2004, 45, 749-52.

4) LB. Forbes, **Appleyard, GD.**& AA. Gajadhar. Comparison of an ELISA using synthetic tyvelose antigen with an ELISA using excretory-secretory antigen for the detection of trichinellosis in swine. Journal of Parasitology 2004, 90, 835-840.

5) Hanik HLJ, Loewen ME, **Appleyard GD**, Grahn BH, Forsyth GW. Differences between expression of mRNA in retinal pigment epithelium of normal dogs. Can J Vet Res. 2004 68, 201-207.

6) KS Janardhan, **GD Appleyard** and B Singh. Expression of integrins alpha-v and beta-3 in acute lung inflammation. Histology and Cell Biology 2004,121, 383-390.

Submitted

1) JL Davies, JA Sibley, EG Clark and **GD Appleyard**. Histologic and genotypic characterization of 30 cases of feline cutaneous mycobacteriosis. Submitted to Veterinary Dermatology 2004.

2) F Girard, I Taranu, IP Oswald, P Hélie, **Appleyard, G.D.**, J Harel & JM Fairbrother. Host immune status is a key determinant in the development of attaching and effacing lesions in weaned pigs. Submitted to Infection and Immunity. 2004.

In Preparation
1) KA McIntosh, JCS harding, JA Ellis, & **GD Appleyard**. Nested PCR detection and duration of porcine circovirus type 2 in semen samples from naturally infected boars.

2) KA McIntosh, JCS harding, JA Ellis, & **GD Appleyard**. Detection of porcine circovirus type 2 viremia and seroconversion in naturally infected pigs in a commercial farrow-to-finish barn.

PROJECT PHOTOS